Diagnosis of metabolic bone disease

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Preface

The main aim of this book is to stress the ways in which radiology, nuclear medicine and biochemistry, either alone or in concert, contribute to the clinical diagnosis of metabolic bone disease. We have also considered in this respect the place and contribution of bone biopsy. The main basis of this book is radiological and as a result there is emphasis on the radiological evaluation, features and differential diagnosis with extensive illustrations. The other modalities present supportive contributions.

The term 'metabolic bone disease' eludes a comprehensively satisfactory definition. It was first introduced by Albright and Riefenstein in 1948 to describe diseases involving the whole skeleton and resulting from disturbances of several responsible factors. These are considered in this book, together with several others which, while not classified strictly as metabolic bone diseases, nevertheless are important differential diagnoses. Likewise we believe that conditions which result from several pharmacological agents in common use which may affect the handling of calcium by the body should also be discussed.

It is obvious that the scope of any book must be limited; as a result certain disorders are considered in greater detail than others. However, the references given at the end of each section will help to fill any deficiency.

Radionuclide techniques have an essential role to play, not only in the diagnostic aspect of these patients but also in their follow-up. Therefore, we decided that the best format to underline this would be for the book to be structured in having radiology, bone biopsy and biochemistry together and a whole chapter devoted entirely to nuclear medicine with relevant cross-references in the rest of the book. The main purpose of the book is to bring to the clinician a compact source of information on these aspects of bone diseases. Most of this information is scattered widely in textbooks of radiology, nuclear medicine and biochemistry and in countless individual papers in medical journals and specialized publications. An earnest effort has been made to assemble much of this material that is relevant and to present it as clearly as possible.

The radiological content of the book is such that we hope it will be of special value to radiologists.

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Contents

CHAPTER ONE

Introduction

With a contribution by L. Harvey

1.1 BONE COMPOSITION

Bone is a highly organized tissue consisting of mineral salts deposited in an organic matrix. Of the total weight of the skeleton, the mineral phase contributes about two-thirds, the remainder consisting predominantly of collagen with small amounts of proteoglycan, lipid and several non-collagenous proteins, including the recently characterized γ-carboxyglutamic acid-containing protein (osteocalcin) and osteonectin. The mineral phase consists of crystals of hydroxyapatite, a complex salt of calcium and phosphate ($Ca_{10}(PO_4)(OH)_2$), and of amorphous calcium phosphate. The skeleton contains 99% of the body's calcium and also 35% of the sodium, 80% of the carbonate, 80% of the citrate, and 60% of the magnesium. Despite the very large preponderance of calcium in the skeleton, fine control of extracellular fluid calcium is achieved and maintained by the interplay of several hormones whose actions will be discussed in more detail.

It is the organizational pattern of the mineral and organic components of bone which determines the successful mechanical function of the skeleton. What is required is a tissue which is light, rigid, of high tensile strength, and not brittle, and this is achieved by a combination of dense, compact bone and cancellous bone, reinforced at points of stress (Glimcher and Krane, 1968; Glimcher 1976).

The active processes of bone formation and resorption which are necessary for normal bone are provided by the major cell types of bone, the osteoblast, the osteocyte and the osteoclast. The *osteoblast* is considered to be the main cell responsible for the synthesis of organic components of bone and its calcification. As will be seen later, there is also now a view that the osteoblast can participate in the control of bone resorption, perhaps by means of intercellular communication processes which are as yet undefined (Rodan and Martin, 1981; Martin, 1983). The osteoblast varies from a tall cuboidal to an almost flattened cell, about 20–30 μm in diameter. It is

mononuclear, with dense peripheral nuclear chromatin and a nucleolus. Its basophilic cytoplasm is rich in endoplasmic reticulum and ribosomes, with a well-developed Golgi apparatus typical of a 'protein factory' cell type. Mitochondria are plentiful and it is rich in alkaline phosphatase. The origin of the osteoblasts is most likely from osteoprogenitors among primitive mesenchymal cells (Owen, 1980), although it has been suggested that they might be derived from the same precursors as capillary endothelial cells. As the osteoblast becomes less active in synthesizing collagen, it flattens out, its alkaline phosphatase activity declines, as does the basophilic nature of its cytoplasm. In this 'resting' form the cells come to line trabeculae, and many are found deeper in bone as *osteocytes*, occupying small cavities within the bone substance and communicating through extensions with neighbouring cells. Although osteocytes do not divide, they are nevertheless active, at least contributing to the transport of mineral and to the dissolution of mineral around their lacunae. Decreased osteocyte numbers are seen in specimens of infarcted bone and their numbers diminish progressively with normal ageing (senile osteoporosis).

Osteoclasts are the major cells responsible for bone resorption. The active osteoclast is a multi-nucleate cell, with a characteristic ruffled border applied to the bone surface. The nuclei may number ten to thirty, and the diameter of the cell varies from 20 to 100 μm. The osteoclasts are rich in lysosomal enzymes, and are phagocytic in their function. When osteoclasts are active at a bone surface, the cytoplasmic membrane is thrown into deep folds and clefts, called a ruffled border, increasing the cell and bone surface available for lytic activity. The multi-nucleate osteoclast is formed by fusion from precursors, and does not undergo further division. The nature of the osteoclast precursor has been a controversial subject for some years. Although it has been considered that osteoclasts and osteoblasts were derived from the same mesenchymal origins, there is now substantial support for the view that osteoclasts arise from a quite different source, i.e. from mononuclear blood cells of the monocyte–macrophage series (Teitelbaum and Kahn, 1980). The implication is that the osteoblasts are the true bone-derived cells, and that osteoclasts are 'wandering' cells which are supposed to carry out the controlled function of removal of mineral and organic constituents of bone. Despite their physical and developmental differences, it is nevertheless clear that the activities of the cells of bone are exquisitely regulated, and that the different cell types rarely, if ever, function independently of each other.

A further cell type, the *endosteal cell*, present on the normal inert surface of all endosteal (inner cortical) and trabecular bone, is a flattened cell with slender nucleus and almost imperceptible cytoplasm. Endosteal cells separate the bone surface from adjacent marrow tissue and may be mistaken for inactive osteoblasts on osteoid or recently mineralized lamellae. Their recognition is important in the accurate quantitative analysis of metabolic disorders. Though potentially osteogenic, they are of little significance in the further consideration of these disorders.

In the adult skeleton, bone is formed in a lamellar arrangement, in which collagen fibres are laid in a parallel, orderly fashion. Osteocytes, which are few, are small and uniform, with their lacunae parallel to the collagen fibres. *Woven bone*, which is seen in the embryo and in pathological states of high bone turnover in adults, consists of a non-parallel, irregular arrangement of collagen fibres, giving a disorganized picture, with many more osteocytes, whose lacunae vary greatly in size. Hydroxyapatite crystals deposit parallel to the collagen fibres in lamellar bone, but in a disorderly array in woven bone.

The lamellar arrangement is essential to the structure of the Haversian system of cortical bone, in which lamellar fibres are arranged concentrically around the central cavity (Haversian canal). From the centre of the Haversian canal there is communication between the osteocytes by means of small connecting channels. Individual Haversian units, called osteons, appear on microradiography to have a circular form. They differ in appearance according to their age and degree of mineralization (Glimcher and Krane, 1968).

The mechanisms of mineralization of bone are not precisely understood. The crystals of hydroxyapatite are deposited parallel to the collagen fibres of matrix, and it is assumed that bone cells (osteoblasts, osteocytes) control the availability of calcium and phosphate at sites of mineralization by transporting the ions themselves, and regulating other activities in the environment. Several components of the ground substance of bone may contribute to the nucleation of mineral salts, including collagen, glycoproteins, proteoglycans and certain non-collagenous proteins, especially the calcium-binding γ-carboxyglutamic acid-containing protein, 'osteocalcin'. In calcifying bone and cartilage, membrane-bound vesicles have been identified which may play a role in the initial mineralization process (Anderson, 1973). Inorganic pyrophosphate (PP_i) is a potent inhibitor of calcification at very low concentrations, and is generated in bone. Fleisch (1974) has proposed that osteoblasts, which are rich in

alkaline phosphatase, could thereby hydrolyse the PP_i, removing its inhibitory influence on mineralization.

Long bones consist of the *diaphysis*, the cylindrical shaft containing the medullary cavity, the *epiphysis*, the most distal part, articulating with adjacent bones, and the *metaphysis* separating the two. Metaphyseal growth is limited in early development by the growth plate. Growth in bone length is determined by proliferation of the cartilage cells of the growth plate, whereas growth in width requires formation of bone at the periosteal surface, and resorption at the endosteal surface, with the rate of formation exceeding that of resorption. Thus the concentric layers of bone in the diaphysis are constantly replaced, while the centrally located ones are destroyed, maintaining the medullary cavity. Growth in length ceases after epiphyseal plate closure occurs. Development of long bones takes place by a process of *endochondral bone formation*, in which new bone matrix replaces calcified cartilage. In addition to this mechanism, in all bones including especially the skull, bone is formed directly from pluripotent connective tissue, by the process of *intramembranous bone formation*.

The process which establishes the architecture of bone has been called *modelling* (Frost, 1973), a process which ceases when bone growth stops (age 18–20). *Remodelling* continues throughout life, involving all surfaces of bone, and is essential to the preservation of normal bone structure. The tight coupling of formation and resorption are required for the success of this, and deviations result in the various metabolic bone diseases. Thus the processes of bone formation and resorption are tightly coupled, with formation equal to resorption rates in normal circumstances. The two processes are maintained at a rapid rate at stages of rapid growth, but slow down appreciably at adulthood. Beginning in the fourth decade of life there is a net slight increase in bone resorption over formation, resulting in a net loss of bone at a rate of about 1% per year in women, slightly less in men. This will be discussed later in relation to the development of osteoporosis.

Resorption of both organic and mineral phases of bone is undertaken chiefly by osteoclasts, with some removal of mineral also evident around osteocytic lacunae. It is not certain whether this 'osteocytic osteolysis' is true resorption.

Several hormones increase osteoclastic bone resorption – parathyroid hormone (PTH), vitamin D, prostaglandins, thyroxine, various growth factors and lymphokines. Calcitonin is the major known hormonal inhibitor of bone resorption. Much less is known of hormonal or other factors capable of stimulating bone formation. Piezo-electric forces generated with movement, and even with the contractile tone

of muscles inserted into bone, provide a necessary stimulus to osteoblastic activity. Removal of this stimulus, e.g. with immobilization, especially complete immobilization as in spinal cord injury, greatly reduces bone formation and results in excess of resorption over formation.

1.2 HORMONES INFLUENCING BONE AND CALCIUM METABOLISM

Understanding of metabolic bone diseases requires an appreciation of the ways in which several hormones act in concert to regulate bone metabolism.

1.2.1 Parathyroid hormone

Parathyroid hormone (PTH) has been known for many years to increase the serum calcium concentration. Albright's earliest view of the mechanisms of action of PTH was that its primary action was upon the kidney to promote phosphorus excretion, thereby depleting the extracellular fluid of phosphorus, which was met in turn by mobilization of calcium and phosphorus from bone (Albright and Ellsworth, 1929). This view was contested by others, including Selye, who showed that removal of the kidneys in rats was followed within 24 hours by an increase in the numbers and activities of osteoclasts in bone, a response which could be prevented by removal of the parathyroid glands. The ability of parathyroid tissue to resorb bone directly was shown first in 1948 and subsequently organ culture data from many workers using purified PTH have established the resorptive effect of the hormone.

The ability of PTH to resorb bone mineral and matrix provided the basis for the McLean and Urist (1955) view of the regulation of plasma calcium which proposed that the level was controlled by a negative feedback mechanism through appropriate changes in the secretion rate of PTH. Rasmussen (1961) pointed to the additional role of the kidney as a fine regulator, capable of rapid adjustment, and responding to PTH by restricting calcium excretion. He included this sensitive regulator with the high capacity, insensitive skeletal system, in a proposed scheme for PTH regulation of the extracellular fluid calcium.

PTH increases the activity and the numbers of osteoclasts in bone. At least one major initial event in PTH action upon its target cells is the stimulation of adenylate cyclase, with increased production of cyclic AMP and activation of cyclic AMP-dependent protein kinases. Such effects of PTH in

bone, together with the ability of analogues of cyclic AMP to resorb bone, pointed to a role for cyclic AMP in mediating the action of PTH on bone resorption. However, it is now clear that the major target cell for PTH effects on cyclic AMP formation in bone is the osteoblast. Indeed there is not yet any evidence for a direct effect of PTH on the osteoclast. This had led to the hypothesis that the osteoblast might mediate the bone-resorbing action of PTH by influencing the recruitment and activity of osteoclasts, perhaps by production of a locally active substance capable of transmitting information between cells. An alternative, or perhaps complementary, mechanism is that PTH acting upon 'resting' osteoblasts, lining trabecular surfaces, could cause them to contract, making the osteoid available for the resorbing action of osteoclasts. Once resorption has started, chemotactic products of resorbed matrix would attract further osteoclasts (Rodan and Martin, 1981; Martin, 1983).

This scheme of involvement of the osteoclast in control of the resorption process fits with known mechanisms of cellular bone resorption. It ascribes to the osteoblast an additional function, which may be allocated to the osteoblast at some particular stage of its maturation. Such a scheme certainly does not imply that osteoblasts do not have a major role in bone formation. Indeed the resorbing hormones almost certainly influence bone formation, and some of them in a biphasic way. Thus for example PTH acutely decreases collagen synthesis in bone *in vitro*, and of course promotes resorption. However, there is increasing evidence *in vivo* that under some circumstances and at certain doses the hormone increases bone collagen synthesis and perhaps bone formation. The cellular actions and communication processes which result in the balanced process of bone remodelling are by no means precisely defined, and work over the next several years should elucidate them. It will be pointed out below, in discussing other resorbing hormones, that they also clearly act on the osteoblast, consistent with the view that they may produce their resorptive effects without necessarily acting directly upon the osteoclast at all.

In summary, PTH is in all respects conservative of the plasma calcium, acting as it does to promote the mobilization of calcium from bone and to restrict calcium excretion via the kidney.

1.2.2 Vitamin D

Vitamin D has a major effect on the skeleton, and its deficiency leads to striking clinical states of metabolic bone

disease. The hypercalcaemia induced by vitamin D was for many years thought to be due to its intestinal effect of increasing calcium and phosphate absorption. However, even after evidence was obtained that a bone effect might operate, demonstration of this took several years to be accomplished. This required the discovery that vitamin D itself needed to be transformed in the body to biologically active forms. The first step, 25-hydroxylation, takes place in the liver (Lund and DeLuca, 1966), and the second major step of 1-hydroxylation takes place in kidney mitochondria to produce the major circulating form of vitamin D, 1,25-dihydroxy-vitamin D ($1,25(OH)_2$ vitamin D). The latter acts directly on bone to promote its resorption, and indeed on a molar basis is the most potent of all resorbing hormones. It is about one thousand times more potent in this respect than $25\text{-}(OH)_2$ vitamin D which is in turn more potent than any other metabolite of the hormone. The same relative potencies exist in effects of vitamin D metabolites on intestinal calcium and phosphate absorption, and in their effects on other target cells. The parent vitamin D itself of course has no significant direct biological action in any of these systems.

Like the other stimulators of bone resorption, $1,25(OH)_2$ vitamin D increases the number and activities of osteoclasts, and furthermore, studies with isolated osteoblast-rich cells of rat and mouse, and with osteogenic sarcoma cells show clearly that specific, high-affinity receptors for $1,25(OH)_2$ vitamin D exist in osteoblasts, whereas it is not yet known whether they exist in osteoclasts. This is again consistent with the idea that the resorbing hormones act first on the osteoblast.

Whatever the significance of the vitamin D effect on bone resorption, there is little doubt that the vitamin-hormone has a crucial role in promoting calcium and phosphorus absorption by the small intestine. It is required for the adequate growth and mineralization of the skeleton, and vitamin D deficiency in growing children results in rickets, characterized by wide unmineralized epiphyseal cartilage plates. In adults there is no cartilage overgrowth, but accumulation of unmineralized osteoid on trabecular and cortical bone surface.

Thus vitamin D makes calcium and phosphate available for the normal mineralization of bone. No more direct effect of any vitamin D metabolite on bone formation has been demonstrated, despite considerable research effort in this area. Furthermore it is not yet certain that the $1,25(OH)_2$ vitamin D effect on bone resorption reflects a physiological role of the hormone. The great sensitivity and specificity of this effect, together with the observation that patients treated

with calcitriol can become hypercalcaemic at doses just above physiological replacement level, do suggest that the hormone might have such a role in addition to enhancing calcium absorption. At all events it is clear that $1,25(OH)_2$ vitamin D has a major role in mineral metabolism, of promoting calcium and phosphate absorption, and probably in some circumstances of promoting bone resorption.

1.2.3 Prostaglandins

Prostaglandins are 20-carbon fatty acid products of the metabolism of arachidonic acid via the cyclo-oxygenase pathway. Virtually all mammalian cells have the capacity to produce prostaglandins, and these molecules are important local regulators of biological activity in many organ systems in the body. There is increasing evidence that this pertains in bone. Klein and Raisz (1970) showed that prostaglandins were potent resorbers of bone *in vitro*, an observation confirmed by many groups. There are many similarities between the actions of prostaglandins and PTH upon bone. They each increase cyclic AMP production in osteoblasts and with each of them there is an increase in the activity and numbers of osteoclasts. Prostaglandin E_2 (PGE_2) is the most potent bone-resorbing prostaglandin. The major difference between PTH and PGE_2 and their actions upon bone is that PTH is clearly capable of elevating the serum calcium when administered to animals whereas this is not so with PGE_2. Prostaglandins are metabolized in the tissues which produce them and in virtually all organs, but particularly the lungs and liver. This breakdown into inactive metabolites occurs rapidly, and an intravenously administered dose of a prostaglandin is cleared almost completely by one circulation through the pulmonary vascular bed. This probably explains the considerable difficulty which has been experienced in raising blood calcium in experimental animals by infusing PGE_2. These observations are relevant to any consideration of a role of prostaglandins as circulating agents acting upon bone. In general it seems more likely that they are important as local mediators. Indeed several agents have been found to promote bone resorption by a mechanism which involves the synthesis within bone of prostaglandins. These include complement-sufficient serum, and certain growth factors including epidermal, fibroblast and platelet-derived growth factors. It is uncertain whether these growth factors function physiologically like circulating hormones, but the strong possibility exists that they are produced locally and have their effects on adjacent bone (Martin, 1983). Two other proteins

found on bone which may be of physiological importance are 'skeletal coupling factor', produced by resorbing bone and suggested to stimulate the osteoblasts to begin bone formation, and 'bone-derived growth factor', also produced by resorbing bone and capable of stimulating osteoblast growth.

1.2.4 Osteoclast-activating factor (OAF)

OAF is a lymphokine product of lectin-transformed lymphocytes and of malignant white blood cells. Like the major bone-resorbing hormones discussed above it increases the activity and numbers of osteoclasts. OAF is probably important in the hypercalcaemia associated with haematological tumours, especially multiple myeloma, and it is possible that this or related lymphokines may be of physiological importance. It is uncertain yet whether the primary direct target cell of OAF in bone is the osteoblast or osteoclast. Excess of OAF could be a determining factor in the development of osteoporosis in multiple myeloma. A further lymphokine which has been shown to promote bone resorption is interleukin 2.

1.2.5 Thyroxine

Thyroxine increases bone resorption, and it is possible that this might contribute to the hypercalcaemia seen in about 30% of patients with thyrotoxicosis, and also to the bone loss which occurs in thyrotoxic patients who remain untreated for prolonged periods.

1.2.6 Calcitonin

Calcitonin is the major hormonal inhibitor of bone resorption. It was discovered as the outcome of experiments in the early 1960s which were carried out because of a dissatisfaction with accepting PTH as the sole mechanism for regulating plasma calcium. Calcitonin was found to lower the blood calcium rapidly in animals, and to do so by inhibiting bone resorption. It was shown to act directly on bone to decrease the numbers and activity of osteoclasts, perhaps by influencing the flow of precursor cells into the osteoclast population and further by direct inhibitory action upon active osteoclasts.

Since calcitonin was found to act by inhibiting bone resorption, it was not surprising that its effect of lowering plasma calcium was most marked under conditions of high bone turnover, such as in the young, growing animal and in pathological states of increased bone resorption in maturity,

but that it had little or no effect on plasma calcium in mature animals or man. There is a view that calcitonin release from the thyroid gland is stimulated during intake of food, and that this hormone release prevents fluctuations in plasma calcium occurring with meals. There is little evidence that this is so in man, and although calcitonin may indeed be a calcium-regulating hormone in stages of high bone turnover, it is possible that in the human adult it remains as a regulator of bone resorption, but that the symptomatic effect of inhibition of bone resorption does not take place because bone resorption is not contributing significantly to the maintenance of the serum calcium.

There is no evidence that calcitonin stimulates bone formation. Indeed coupling between bone resorption and formation is so regulated that when calcitonin is used to inhibit resorption, there follows a decrease in bone formation.

1.2.7 Oestrogenic and androgenic hormones

Both oestrogenic and androgenic hormones have been thought to have a role in normal bone physiology, but most attention has been directed towards oestrogen. The development of osteoporosis after oestrogen withdrawal has been recognized for many years, and has provided the impetus to the study of oestrogen effects. No convincing direct effects of oestrogen on bone have been demonstrated. The suggestion that oestrogen inhibited the bone-resorptive effect of PTH was obtained at very high oestrogen concentrations and was not confirmed in other studies. Furthermore many groups have attempted to identify oestrogen receptors in bone and bone cells and have failed to do so. It seems likely that any effect of oestrogen upon bone is indirect, and it will be most important to determine the nature of this effect. One relevant possibility is that oestrogen deficiency might act through calcitonin. Circulating levels of calcitonin are known to be decreased in post-menopausal women, and to be restored to male and to pre-menopausal levels by administration of oestrogen. Thus a possibility is that the effect of oestrogen withdrawal is explained by the consequent withdrawal of calcitonin as an important functional inhibitor of the bone-resorptive process.

1.2.8 Summary

Many peptide and steroid hormones, prostaglandins and growth factors influence the processes of bone resorption and